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WATER-SOLUBLE NATIVE DRY PLANT EXTRACT, IN PARTICULAR GINKGO BILOBA EXTRACT WITH A HIGH CONTENT OF TERPENOIDS AND FLAVONGLYCOSIDES

Background of the Invention

Description

The invention relates to a waters-soluble, native dry extract consisting of plant parts, in particular Ginkgo biloba leaves, and a procedure for its manufacture.

In the following, "water-soluble" means soluble and "readily soluble" as defined in the "European Pharmacopoeia" 1997, Edition (official German edition, Deutscher Apotheker Verlag Stuttgart, Govi-Verlag-Pharmazeutischer Verlag GmbH Eschborn).

Preparations based on Ginkgo biloba leaf extracts are used in a variety of ways in medicine and cosmetics. The pharmaceutical effect of Ginkgo biloba extracts can be attributed primarily to the constituents Ginkgo flavonglycosides and terpenoids (e.g., ginkgolides, bilobalide).

Ginkgo biloba extracts can be manufactured in various ways. In one common basic procedure, Ginkgo biloba leaves are first extracted with an extractant consisting of an aqueous solution of a low-aliphatic ketone or an alcohol. The resulting raw extract is subjected to precipitation with water in the cold for purification purposes, and the precipitated waste products, in particular lipophilic constituents, are removed.

Different procedures are known in prior art to further purify this raw extract with the objective of enriching desired constituent groups. Lead precipitation is described in DE 39 40 091 C2, for example, resulting in the removal of many undesired components, but bringing with it the disadvantages associated with the use of lead, in particular a health risk to persons working with the lead and relatively high costs.

DE 39 40 092 C2 proposes an extraction of the primary extract with n-butanol in water instead of lead precipitation, while US 5 637 302 suggests an extraction with n-butanol and toluene. These measures are associated with the disadvantage of using organic solvents that might potentially constitute a health hazard.

In J 27 93 00/1994, the raw extract is adsorbed on polar adsorber resins to enrich the desired valuable products.

In all of these described extracts, constituents of relevance in terms of pharmacological efficacy are enriched, normally in such a way that the ratio of drug to extract measures 30 - 70 to 1. The Gingko biloba dry extracts resulting from this manufacturing procedure all exhibit poor water solubility. For this reason, these extracts are often subjected to additional treatment to improve their known poor water solubility. To this end, various procedures are also known in prior art, but all of them represent only compromise solutions.

EP 0 764 659 A1 describes a procedure for improving the extremely low water solubility of the ginkgolides, which are especially important from a therapeutic standpoint (solubility under 0.02 %), characterized by the execution of a complexing reaction between the ginkgolides and cyclodextrins, and leading to ginkgolide-cyclodextrin complexes that readily enter into solution with water. However, this complexing procedure is very complicated from a technical standpoint.

DE 43 34 600 C2 discloses the use of dimethyl isosorbide and polyalcohol as a solubilization aid for Ginkgo biloba extracts in aqueous solution or a water-oil emulsion.

To improve the water solubility of difficultly water soluble flavonoids, EP 0 577 143 A2 generally proposes that the flavonoids be distributed in a molecularly disperse manner in a basic substance consisting of hydrophilic peptide with a molecular weight exceeding 100 Daltons, especially gelatins, and in so doing keep them in a stable solid or liquid solution.

An alternative procedure is described in EP 0 275 005, namely the conversion of flavonoids with phospholipids as the solubilization agent.

Finally, WO 96/29085 discloses a Ginkgo biloba dry extract preparation containing as a solubilization agent an effervescent mixture of a physiologically compatible acid

or its sodium salt and a physiologically compatible carbonate or hydrogen carbonate.

The mentioned procedures from prior art are all associated in particular with the disadvantage that special solubilization aids or other galenic aids are used during or after manufacture of the extract. In the end, these aids are also contained in the final formulation of the active ingredient, where they are not always desired or can sometimes even be considerably disruptive (e.g., because they bind the ginkgolides in complexes, thereby impeding their liberation).

An Summary of the Invention

~~The~~ object of this invention is to provide a native dry extract consisting of plant parts, which is completely water soluble and exhibits a high content of relevant constituents, in particular a native, completely water-soluble dry extract consisting of Ginkgo biloba leaves with a high content of terpenoids and flavonglycosides, in which the mentioned disadvantages are avoided, and also to provide a procedure for manufacturing such dry extracts.

This object is achieved by providing a dry extract consisting of plant parts, in particular of Ginkgo biloba leaves, which consists exclusively of plant part constituents, i.e., contains no additional substances relative to the extract composition, and in particular lacks any added solubilization aids.

The dry extract according to the invention contains practically all plant constituents desired from a

pharmaceutical, cosmetic and dietetic standpoint, primarily terpenlactones and flavonglycosides, and can in particular readily contain prodelphinidines and other proanthocyanidins.

In a preferred embodiment, the dry extract according to the invention can also have a significantly higher percentage content of terpenlactones and flavonglycosides in comparison to the leaf(drug).

The dry extract according to the invention can involve both the primary or raw extract obtained from the leaf (drug), as well as a partially or largely purified extract. A partially purified extract can be obtained by removing the extraction solvent from the raw extract, and again diluting the raw extract concentrated in this way by adding water, and by subjecting this aqueous extract solution to a cold treatment for precipitating out undesired, primarily lipophilic constituents. For example, a largely purified extract according to the invention is obtained by purifying in a known manner the residue obtained in the above procedure during the precipitation reaction, e.g., via additional precipitation reactions, adsorption and desorption procedures, extraction with n-butanol, etc. (compare DE 39 40 091, DE 30 40 092, US 5,637,302, J 279 300).

The dry extract according to the invention is readily soluble in water, i.e., it is soluble practically without a trace at a volume ratio of at least 1 part extract to 10 parts water per the "European Pharmacopoeia" 1997. A clear

solution comes about, which remains unclouded even after several hours.

Such a dry extract can not only be used to very good effect in pharmaceutical products, but just as well in cosmetic and dietetic products.

One particularly advantageous variant of a dry extract according to the invention is characterized by the fact that it has a content of:

at least 20 % (m/m) flavonglycosides,
at least 5 % (m/m) terpenlactones and
at most 5 ppm ginkgolic acids.

Another, also very advantageous variant of the dry extract according to the invention is characterized by the fact that it has a content of:

at least 22 - 27 % (m/m) flavonglycosides,
at least 5 - 7 % (m/m) terpenlactones,
at least 2.8 - 3.4 % (m/m) ginkgolides A, B, C,
at least 2.6 - 3.2 % (m/m) bilobalide, and
at most 5 ppm of ginkgolic acids.

This variant reflects the data in the monograph entitled "Ginkgo biloba Dry Extract" put out by Commission E of the former Federal Ministry of Health of the Federal Republic of Germany.

The object underpinning the invention is also achieved with a procedure for manufacturing a dry extract according to the invention. This procedure according to the invention

is characterized by the fact that a liquid extract, preferably a hydroalcoholic liquid extract is initially manufactured in any manner desired, in particular in a conventional way with water or organic solvents or mixtures thereof, if necessary via the indirect method of dry extract production to remove undesired solvents not suited for ultrafiltration and subsequent reintroduction of the dry extract in hydroalcoholic solution or another of the mentioned solvents, and that this liquid is then subjected to targeted ultrafiltration. Use is preferably made of filters consisting of polyamide, polypropylene or regenerated cellulose, each with an average pore size ranging from 2000 to 10000 Daltons. The use of filters with roughly a 3000 Dalton pore size is especially preferred. Organic solvents are forcibly removed from the liquid ultrafiltrate, which is then dried as well, if desired. Without being subjected to a final drying stage, the ultrafiltrate minus the organic solvents can be used directly for further processing in pharmaceuticals, cosmetics and/or dietetic foodstuffs.

Therefore, the easiest way to obtain the dry extracts according to the invention described above is to subject raw extracts or partially or largely purified extracts to an ultrafiltration procedure in any manner desired and then drying them. In other words:

The dried extract according to the invention can be obtained by subjecting a primary or raw extract manufactured in a conventional manner to ultrafiltration

and then removing the organic solvent(s) and, if necessary, drying the ultrafiltrate.

The dry extract partially purified in comparison to the raw extract can be obtained by removing the extraction solvent from the raw extract, diluting the raw extract concentrated in this way by adding water, then subjecting the aqueous solution to cold treatment to precipitate out lipophilic constituents, if necessary react the residue with alcohol or another organic solvent suitable for ultrafiltration to improve the dissolving behavior of the extract substances, subsequently subjecting this solution to ultrafiltration and finally removing the organic solvent(s) and, if necessary, drying the ultrafiltrate.

The dry extract largely purified in comparison to the raw extract can be obtained via the following steps: Removing the extraction solvent from the raw extract, diluting the raw extract concentrated in this way by adding water, cold treatment to precipitate out lipophilic constituents, removing undesired constituents from the residue via precipitation reactions, performing adsorption and desorption procedures, extracting with n-butanol, or similar purification procedure, if necessary drying the extract purified in this way to remove solvents not suited for ultrafiltration, reintroducing the dried extract in preferably a hydroalcoholic solution, and subsequently subjecting this preferably hydroalcoholic liquid extract to ultrafiltration and finally removing the organic solvent(s) and, if necessary, drying the ultrafiltrate.

The invention is based on the completely unexpected finding that extract components that impede the water solubility of dry extracts can evidently be removed or at least deactivated just by targeted ultrafiltration, while the composition of the desired constituent groups of the extract remains essentially unchanged. In the case of Ginkgo biloba dry extracts, ultrafiltration causes even those ginkgolides regarded as difficultly soluble to completely dissolve in water.

The combination of extract properties according to the invention, namely water-soluble, native, consisting exclusively of plant part constituents and in particular free of solubilization agents and/or galenic aids, can evidently be brought about solely via ultrafiltration treatment.

The fact that a purely technical step can yield a dry extract completely soluble in water is all the more astounding, since previous solubility improvements for dry extracts could only be achieved by adding galenic aids or solutizers.

The ultrafiltrate freed of organic solvents can also be used directly according to the invention, i.e., without subsequent drying, for further processing, e.g., in pharmaceuticals, cosmetics and/or dietetic foodstuffs.

In the following, the invention will be explained in greater detail based upon a graphic depiction on Fig. 1 and through the use of embodiments.

Based on the example of Ginkgo biloba, Fig. 1 shows the possible methods for manufacturing Ginkgo biloba dry extracts having the most varied of purity levels.
The drug in the form of fresh or dried Ginkgo biloba leaves is the starting material in each case.

A first extract, the primary or raw extract, is manufactured out of these leaves using a hydroalcoholic or hydroketonic solvent.

This raw extract can already be subjected to ultrafiltration and converted into a water-soluble dry raw extract through subsequent drying (method 1).
In many cases, however, it is necessary or desirable to remove unwanted constituents from the raw extract before using it for its intended purpose. To this end, (much of) the extraction solvent(s) is/are generally removed from the extract first, the concentrated raw extract is diluted again by adding water, and this aqueous mixture is subjected to a precipitation reaction via cold treatment, during which primarily lipophilic constituents are precipitated and separated out. This partially purified, liquid extract can then be subjected to ultrafiltration and then dried, yielding a water-soluble, partially purified dry extract (method 2).

However, to manufacture relatively pure extracts in which undesired constituents have been largely removed and desired constituents have been enriched, this pre-purified or partially purified liquid extract is subjected to additional purification procedures, e.g., precipitation reactions as described in DE 39 40 091, or extraction

procedures with n-butanol as described in DE 30 40 092 and US 5,637,302, or adsorption and desorption procedures as described in J 279 300. The largely purified extracts obtained with this purification procedure can then be subjected to ultrafiltration and then dried, either directly if present as liquid extracts and the solvent in question is suitable for an ultrafiltration procedure (method 3), or indirectly, specifically by first concentrating and/or drying to remove unsuitable solvents and subsequently reliquefaction through introduction in a preferably hydroalcoholic solution (method 4). The end product of methods 3 and 4 is a water-soluble, largely purified dry extract according to the invention. The "previous" method shown parallel to method 4 in the figure illustrates the exceeding simplicity of the procedure according to the invention by comparison to the procedure known from prior art for obtaining largely purified dry extracts soluble in water.

dn s92 dn s93 ~~Embodiments~~

In all examples described below, the used starting extracts can be manufactured in various processes, in particular with various extractants, e.g., acetone, ethanol or butanol.

Example 1:

4.65 g of Ginkgo biloba EGb 761 dry extract are set to a 10 % solution with 50 (m/m) % ethanol and filtered in an ultrafiltration system using a polyamide membrane with a pore size of 5000 Daltons. The retentate is again rinsed

six times with 30 ml of 60 (m/m) % ethanol. The solution traversing the filter (= filtrate) is concentrated (e.g., under a vacuum in a rotary evaporator) and dried overnight in a vacuum drying cabinet at 45 °C and < 50 mbar.

Obtained as a final result are 3.47 g of filtrate (= 74.62 % yield) and 1.18 g of retentate (= 25.38 % yield).

Extract analysis revealed the following contents:

	Determined values (Example 1)
Total terpenlactones	6.3 %
Flavonglycosides	24.05 %
Ginkgolic acids	< 5 ppm

The dry extract from the filtrate can be completely and clearly dissolved as a 0.1 % solution in water. The solution does not cloud up while standing for a 2 hour period. Filtering the solution through a filter layer (paper filter, pore size 1 μm) results in only a very slight filter residue of 0.12 % of the weighed extract portion (= dry extract in the solution).

Example 2:

1.3 kg of Ginkgo biloba leaves with 1 % flavonglycosides and 0.26 % terpenlactones are extracted twice by means of vortex extraction with a total of 10.5 kg of 80 (m/m %) ethanol at 60 °C. The raw extract solution with the dissolved constituents is separated from the extracted drug parts using a vacuum nutsche and a Seitz No. 1500 plate filter.

This results in 9.5 kg of filtered raw extract solution with a solids content of 3.85 %. The solution is gently concentrated under a vacuum at a max. product temperature of 65 °C in a ratio of 12 to 1 to a concentrate with 44 % dry residue.

The concentrate is set to 17 % solids content while adding demineralized water, and then cooled to 8 °C overnight. The precipitated water-insoluble constituents are filtered out through a 1500 Seitz plate filter while adding 107.6 g of filter aid ("Filter Cel", Lehman & Voss & Co., Hamburg).
This results in a clear extract solution of 2.4 kg with a solids content of 10 %.

The extract solution is pumped through a column with 0.96 liters of adsorber resin Diaion HP20 from Mitsubishi Chemical. After charging the extract, the column is rinsed with 1.6 liters of demineralized water and 3 liters of 60 (m/m) % ethanol.

The 60 (m/m) % ethanol desorbate in the column with a solids content of 1.6 % (48 g dry of dry extract) is directly filtered using an ultrafiltration system with a polypropylene membrane having a pore size of 5000 Daltons (Dow Danmark). The membrane is rewashed five times with 500 ml of 60 (m/m) % ethanol. The resulting filtrate is gently concentrated under a vacuum and dried overnight in the drying cabinet at 45 °C at < 50 mbar. 36.44 g of dry

extract are obtained in the filtrate, which corresponds to a yield of 2.8 % relative to the used quantity of drug.

Extract analysis:

	Determined values (Example 2)
Total terpenlactones	6.95 %
Total ginkgolides A, B, C	3.51 %
Bilobalide	3.44 %
Flavonglycosides	26.73 %
Ginkgolic acids	< 5 ppm

The water solubility test involves dissolving 1 part extract into 10 parts water while mixing. The extract spontaneously dissolves clear, and can be defined as "readily soluble" according to the "European Pharmacopoeia" 1997. Filtering the solution over a filter layer (paper filter, pore size 1 μm) results in only a very slight filter residue of 0.18 % of the weighed extract portion (= dry extract in solution). Prior to ultrafiltration, the extract is difficultly soluble as defined in the "European Pharmacopoeia" 1997.

Example 3:

98 g of the extract used in 2.2 are dissolved in 1000 ml of 60 (m/m) % ethanol, and filtered using a spiral cartridge S1 Y3 (Amicon) consisting of regenerated cellulose having a pore size of 3000 D. The membrane is rewashed twice with 500 ml of the same solvent.

The resulting filtrate is then gently concentrated under a vacuum and dried overnight in the drying cabinet at 45 °C and < 50 mbar. 76.37 g of dry extract are obtained, corresponding to a yield of 77.9 %.

Extract analysis:

	Determined values (Example 3)
Total terpenlactones	6.77 %
Total ginkgolides A, B, C	3.49 %
Bilobalide	3.28 %
Flavonglycosides	26.02 %
Ginkgolic acids	< 5 ppm

Water solubility is defined as "readily soluble" according to the "European Pharmacopoeia" 1997 (see Example 2). Filtering the solution over a filter layer (paper filter, pore size 1 µm) results in only a very slight filter residue of 0.2 % of the weighed extract portion (= dry extract in solution).

Example 4:

100 g of a Ginkgo biloba dry extract manufactured as instructed in German Patent DE 39 40 092 are dissolved in 1000 ml of 60 (m/m) % ethanol and ultrafiltrated using a polypropylene membrane having a pore size of 10000 Daltons. The membrane is rewashed twice with 1000 ml of the same (aforementioned) solvent. The resulting filtrate is gently concentrated under a vacuum and dried overnight at a temperature of 45 °C and pressure of < 50 mbar. 82.64 g of extract are obtained, whose content of terpenlactones, ginkgolides A, B and C, bilobalidene, flavonglycosides and

ginkgolic acids lies within the respective range defined in the monograph entitled "Ginkgo biloba Dry Extract" put out by Commission E of the former Federal Ministry of Health of the Federal Republic of Germany.

6 g of this dry extract are dissolved in 50 g of demineralized water. Water solubility is defined as "readily soluble" according to the "European Pharmacopoeia" 1997. Filtering the solution over a filter layer (paper filter, pore size 1 µm) results in only a very slight filter residue of 0.63 % of the weighed extract portion (= dry extract in solution).

Example 5: Control Tests

a) 5 g of Ginkgo biloba dry extract EGb 761 are dissolved in 50 g of demineralized water. Water solubility is defined as difficultly soluble according to the "European Pharmacopoeia" 1997. Filtering the solution over a filter layer (paper filter, pore size 1 µm) results in filter residues averaging 18.2 % of the weighed extract portion (= dry extract in solution).

b) 5 g of Ginkgo biloba dry extract manufactured according to the ethanol method (per JP 279 300) and without ultrafiltration are dissolved in 50 g of demineralized water. Water solubility is defined as difficultly soluble according to the "European Pharmacopoeia" 1997. Filtering the solution over a filter layer (paper filter, pore size 1 µm) results in filter residues averaging 15.2 % of the weighed extract portion (= dry extract in solution).